REMARKS

The Office Action

Claims 1, 4, and 16-32 are pending in this application. All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 25-32 stand rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Claims 16-18 and 30-32 stand further rejected under 35 U.S.C. § 112, second paragraph for including new matter.

Rejections Under 35 U.S.C. § 112, first paragraph

All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner applies a four-part rejection. First, the Examiner asserts that "[a]lthough the preamble implies that the method will result in treatment of the disease, no particular treatment effect is achieved." Second, the Examiner asserts that "[n]either the prior art nor the instant specification teach that serotonergic neurons are useful in treating Parkinson's Disease." Third, the Examiner asserts that "[t]he specification fails to provide an enabling disclosure for the genetic modification of human ES cells." Fourth, the Examiner asserts that the specification fails to enable a method for transplantation of ES cells. Applicants address each basis for rejection separately below.

Therapeutic Effect

In the first part of the enablement rejection the Examiner asserts that "[a]lthough the preamble implies that the method will result in treatment of the disease, no particular treatment effect is achieved." Applicants have addressed this rejection by amendment of the claims.

As amended, all of the pending claims, claims 1, 4, and 16-32, include the limitation "to improve motor function" in the patient, a specific effect produced by performing the method of the invention.

In light of this amendment to the claims, applicants respectfully request that this

part of the lack of enablement rejection be withdrawn.

Serotonergic Neurons

In the second part of the enablement rejection the Examiner asserts that "[n]either the prior art nor the instant specification teach that serotonergic neurons are useful in treating Parkinson's Disease." Applicants have addressed this rejection by amendment of the claims.

Claims 16 and 30 have been amended to remove reference to "serotonergic" neurons.

In light of this amendment to claims 16 and 30, applicants respectfully request that this part of the lack of enablement rejection be withdrawn.

Genetic Modification of Human ES Cells

In the third part of the enablement rejection the Examiner asserts that "[t]he specification fails to provide an enabling disclosure for the genetic modification of human ES cells." The rejection essentially turns on the Examiner's contentions that (1) the specification only teaches transfection of human ES cells using an adenoviral vector and (2) low transfection efficiency would prevent one from being able to perform the claimed methods. Applicants respectfully disagree and address this rejection with the following remarks.

The specification teaches a variety of methods for achieving transfection

The specification provides ample guidance for using a variety of transfection methods to produce genetically modified human ES cells, including electroporation and chemical transfection. See, for example, page 10, lines 6-12, where the specification recites:

> The recombinant molecule may be introduced into the TESCs or the cells differentiated

from the stem cells using in vitro delivery vehicles or in vivo techniques. Examples of delivery techniques include retroviral vectors, adenoviral vectors, DNA virus vectors, liposomes, physical techniques such as microinjection, and transfection such as via electroporation, calcium phosphate precipitation, or other methods known in the art for transfer of creating recombinant cells.

Also, murine ES cells were successfully transfected with a vector encoding Nurr-1 using Lipofectamine according to the manufacturer's protocol (see Examples 2, 9, and 10).

Applicants note that the specification is not limited in its teaching by the presence of the specific embodiment of Example 6. Nowhere in the specification is it stated that one must use an adenovirus vector to achieve transfection of human ES cells.

Finally, the examiner concedes that Eiges *et al.* (*Curr. Biol.* 11: 514-518, 2001) demonstrates that Lipofectamine was a successful, albeit inefficient, method for transfection of human ES cells (Office Action at page 5). Because even a very inefficient transfection process can be used to practice the claimed invention, see below, the genetic modification of human ES cells as applied to the present invention is fully enabled.

Inefficient transfection
is sufficient to practice the
claimed methods

The specification provides ample guidance for selectively isolating homogenous cell populations. See, for example, the specification from page 3, line 21, to page 4, line 12, which recites (emphasis added):

From differentiated ES cells, homogeneous cell populations of specific neuronal cell-fate are isolated by inserting a selectable marker gene cassette into a cell-specific gene expressed in a specific neuronal cell-type. Homogeneous cells or defined heterogeneous cell populations that can be reliably obtained and generated in sufficient numbers for a standardized medically effective intervention are also featured in this invention. For example, inserting a selectable gene cassette, e.g., b-geo (encoding for both neomycin resistance and b-galactosidase) into the dopamine transporter (DAT) or the tyrosine hydroxylase (TH) gene allows the selective isolation of DA neurons. These pure DA neurons are a useful source of donor cells for grafts into PD patients. . Likewise, one can isolate serotonergic neurons from differentiated ES cells by inserting the same b-geo gene cassette into the tryptophan hydroxylase or the serotonin transporter gene that is expressed by serotonergic neurons or isolate astrocytes by inserting the b-geo gene cassette into the fibrillary acidic protein gene expressed by astrocytes. Furthermore, other nerve cells or glial cells can be similarly targeted for lineage restricted populations derived from

embryonic stem cells. Specific lineage-restricted neural precursors thus can be isolated and expanded as a pure population, and used as donor cells in transplantation therapy of different neurological diseases, disorders, or abnormal physical states.

Because specific lineage-restricted neural precursors can be isolated and expanded as a pure population, the efficiency of transfection is irrelevant to the enablement of the claimed methods. Thus, even if it were necessary to engraft exclusively transfected cells, all that is required to practice the present invention is that some transfection of ES cells is achieved, however inefficient, followed by isolation and expansion of the transfected cells.

The art cited by the Examiner demonstrates that the specification, combined with techniques and reagents available at the time of filing, enables the genetic modification of human ES cells. In view of these arguments, applicants respectfully request that this part of the lack of enablement rejection be withdrawn.

Transplantation of ES Cells

In the fourth part of the enablement rejection the Examiner asserts that the specification fails to enable a method for transplantation of ES cells. The rejection essentially turns on the Examiner's contention that though transplantation dopaminergic neurons is known in the art, the transplantation of ES cells is not known or was unpredictable at the time of filing. Applicants respectfully disagree and address this rejection with the following remarks.

The therapeutic effect is conferred by the cell, not by the gene

The expression of a cell fate-inducing gene by the ES cell is merely to promote differentiation along a dopaminergic cell fate pathway and is performed primarily in culture prior to transplantation. As demonstrated in Figure 5B (also see Example 11, page 32), Nurr-1 expression *in vitro* urges a higher proportion of cultured ES cells to

adopt a dopaminergic phenotype, compared to cultured naïve ES cells.

The dopaminergic-inducing effect of expressing a cell-fate inducing gene *in vitro* is complemented by transplantation of the ES cell into the brain. As demonstrated by Deacon *et al.* (Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. *Exp. Neurol.* 149: 28-41, 1998; art of record) and in Example 13 (page 35), naïve ES cells differentiate along a dopaminergic cell fate pathway upon transplantation into the brain. Thus, the transplanted ES cells differentiate *in vivo* regardless of whether they express a cell fate-inducing gene. Contrary to the Examiner's assertion, it is the cell, not the gene, which confers the therapeutic effect and, because the phenotypic change in the ES cell can be monitored *in vitro* prior to transplantation, no guidance on *in vivo* gene expression is required.

The ES cell compositions
described in the specification
become functioning neurons
upon transplantation

The specification demonstrates that naïve ES cells spontaneously adopt a neuronal phenotype when transplanted into the brain (see Examples 12-14). Dopaminergic differentiation of naïve ES cells was observed in two different rodent models of Parkinson's disease—the MPTP mouse model (Examples 12 and 13) and the 6-hydroxydopamine rat model (Examples 14 and 15).

The specification provides significant teachings on the number of ES cells that should be used for transplantation. Applicants direct the Examiner's attention to Example 13; an experiment characterizing the effect of low density and high density ES cell grafts on dopaminergic differentiation in the MPTP mouse model of Parkinson's disease. In this experiment, applicants engrafted 50,000, 2,000, or 200 naïve ES cells into the lesioned striatum and discovered that the best result was achieved with the lowest density graft. Specifically, grafts containing 200 ES cells resulted in an average of 1250 dopaminergic neurons and an absence of tumor-like formations. The 2,000 ES cells

grafts produced fewer dopaminergic neurons while the 50,000 ES cell grafts produced tumor-like structures—an adverse effect. Thus, applicants have demonstrated that low density ES cell grafts are superior to high density ones. Here again, only routine experimentation is required to optimize the number of ES cells that, when engrafted into to a human brain, will give the most beneficial result.

The specification demonstrates that transplantation of ES cells improves motor function

Applicants also demonstrate the therapeutic efficacy of ES cell transplants. In Examples 14 and 15, and Figure 7, applicants demonstrate that naïve ES cell grafts containing 1,000-2,000 cells are sufficient to reduce the functional deficits caused by dopaminergic denervation in the 6-hydroxydopamine model of Parkinson's disease. Specifically, amphetamine-induced turning (a functional indicator of a unilateral dopaminergic deficit) is reduced in rats 7 and 9 weeks after transplantation of the ES cells into the lesioned striatum.

Taken together, the data presented in the specification provides the skilled artisan with ample guidance on methods for ES cell transplantation, including the number of cells to be engrafted and an indication of the expected onset of therapeutic benefit. The rodent models used in these experiments are well validated in the prior art as surrogates for Parkinson's disease in humans. In view of these arguments, applicants respectfully request that this part of the lack of enablement rejection be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 25-32 stand rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. The Examiner has rejected claims 25-29 in their recitation of "recombinant embryonic stem," claims 26-27 in their recitation of "said stem cells or are transfected," claims 26-28 in their recitation of "said stem cells," claim 29 in its recitation of "said recombinant cells," and claims 29-32 in their recitation of "derived from."

Applicants have addressed each of these rejections by amendment of the claims. Specifically, claim 25 has been amended to recite "recombinant embryonic stem cell," claims 26 and 27 have been amended to recite "said stem cells are transfected," claim 29 has been amended to recite "said stem cells," and claims 29 and 30 have been amended to recite "cultured from." In view of these amendments, applicants request withdrawal of these indefiniteness rejections.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 16-18 and 30-32 stand further rejected under 35 U.S.C. § 112, second paragraph for including new matter. The Examiner has rejected claims 16-18 and 30-32 in their recitation of "50 to 50,000 cells per microliter." Applicants address this rejection with the following remarks.

Claims 16 and 30 have been amended to recite the range "100 to 50,000 cells per microliter." Support for this range is found in the specification at page 35, lines 17-18.

In light of this amendment to claims 16 and 30, applicants respectfully request that the new matter rejection be withdrawn.

Support for Amendments to the Claims

All of the pending claims, claims have been amended to include the limitation "to improve motor function." Support for improved motor function is found in the specification at page 40, lines 10-14, and in Figure 7, which shows ES cell-associated restoration of DA dependent motor function in an animal model for Parkinson's Disease. Support for a therapeutic amount of cells to engraft is found in the specification from page 11, line 15, to page 12, line 24. No new matter has been added with this amendment.

CONCLUSION

Applicants submit that the claims are now in condition for allowance and such action is respectfully requested. To expedite prosecution applicants request a telephonic interview with the Examiner to discuss any remaining rejections. The Examiner is invited to call the undersigned at 617-428-0200.

Enclosed is a petition to extend the period for replying for three months, to and including October 2, 2005. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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